AN INTERIM METHOD FOR THE DETERMINATION OF ASBESTOS FIBRE CONCENTRATIONS IN WATER BY TRANSMISSION ELECTRON MICROSCOPY

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Ontario
Ministry of the Environment

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PREPARED BY

THE COMMITTEE ON ASBESTOS ANALYSIS

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FOREWORD

Asbestos is an ubiquitous pollutant, known to be hazardous to health and its presence in the environment is a cause for concern.

Attempts to quantify asbestos in water supplies in Ontario have been hampered because widely divergent results have been obtained by different laboratories analyzing identical water samples. The procedures used by the laboratories were found to differ in the manner of sample preparation and also with respect to the criteria used for fibre enumeration and identification.

To resolve the difficulties associated with the use of diverse methodologies, the Ontario Ministry of the Environment in 1976 established and funded a Committee on Asbestos Analysis, to examine the methods in use and to reach a consensus on an optimum state of the art method, suitable for the routine analysis of asbestos in water.

The method described in this report, "An Interim Method for the Determination of Asbestos Fibre Concentrations in Water by Transmission Electron Microscopy", is that recommended by the Committee.

The results of tests and analyses of data upon which the selection and adaptation of the method is based are presented in a separate Ministry report, "Inter-Laboratory Comparison of Selected Methods for the Determination of Asbestos Fibre Concentrations in Water".

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AN INTERIM METHOD FOR THE DETERMINATION OF ASBESTOS FIBRE CONCENTRATIONS IN WATER BY TRANSMISSION ELECTRON MICROSCOPY

1. Scope

1.1 The method is designed to determine the number and mass of chrysotile and amphibole asbestos fibres per unit volume and the fibre lengths and widths, in samples obtained from drinking water, water supplies and other waters of relatively low total suspended solids content. In general, the method will detect fibres having lengths of approximately 0.2 μm and greater.

1.2 The method is considered to be tentative only and as such is subject to revision as further knowledge on the determination of asbestos is gained.

2. Summary of Method

2.1 A known volume of the water sample is filtered through a membrane filter in such a manner as to distribute the particulate material, including asbestos, uniformly on the filter surface. A thin coating of carbon is evaporated onto the particulate material on the filter. A small section of the carbon-coated filter is placed on a transmission electron microscope grid in a modified Jaffe wick washer and the filter is dissolved by means of chloroform. The prepared grid is examined in a transmission electron microscope and the asbestos fibres are identified, counted and sized at a magnification of 20,000X. Chrysotile fibres may be identified by their morphology only, subject to confirmation of the identity of a portion of the fibres by their morphology and selected area electron diffraction (SAED) patterns. Amphibole fibres are identified by their morphology and SAED patterns. Concentrations are reported in terms of million fibres per litre and in micrograms per litre, for chrysotile, amphibole and total asbestos.

2.2 Samples which contain an excessive amount of organic material are treated in a low temperature oxygen plasma system. The ash remaining is then dispersed in filtered distilled water in an ultrasonic bath to obtain a suspension for filtration.

2.3 The preparative analytical steps can be completed in approximately 24 hours. A batch of several samples may be prepared at one time. The average time required to examine a sample in the electron microscope is approximately 3 hours.

3. Definitions

Asbestos - a generic term for several fibrous silicate minerals of the serpentine or amphibole groups. Examples are, chrysotile, actinolite, cummingtonite-grunerite, anthophyllite, crocidolite and tremolite.

Fibre - a particle having essentially parallel sides and a length to width ratio of 3:1 or greater. An asbestos fibre may be an individual fibril or a bundle of fibrils.

Fibril - a single fibre, which cannot be separated into smaller components without losing its fibrous properties or appearance.

Aspect Ratio - the ratio of length to width.

Chrysotile - a fibrous hydrated magnesium silicate mineral of the serpentine group, consisting of curved layers which overlap to form either scrolls or concentric cylinders, usually exhibiting a tubular appearance when viewed in the transmission electron microscope. The average fibril diameter is about 300 Å.

Amphibole - a double chain silicate consisting of Si_4O_{11} units, laterally linked by various ions such as calcium, magnesium, iron, aluminum and sodium. Amphiboles may consist of or contain fibres formed through natural growth processes and may also produce fragments that

conform to the definition of a fibre as a result of crushing and milling processes.

4. Range

4.1 A range of concentration of about 0.1 million to 1000 million asbestos fibres per litre can be determined without resorting to dilution of the original water sample.

5. Detection Limit

5.1 The minimum detection limit of the method for a given sample depends chiefly upon the blank level for the sample and the presence of extraneous particulate material in the sample. Because of the variability encountered in blank levels, the detection limit is taken to be twice that of the blank level. If the blank level is found to be zero, the detection limit is taken to be 1 fibre in the number of electron microscope grid openings examined. The detection limit for a given analysis can be expressed as million fibres per litre by using the appropriate conversion factors. As an example, a detection limit of 0.2 million fibres per litre may be achieved if sufficient sample volume is used and a blank level of two fibres in twenty grid openings (200 mesh grid) can be obtained.

6. Precision and Accuracy

6.1 Based upon the analysis by six laboratories of filters prepared from four water samples, the inter-laboratory precision of the method, expressed as relative standard deviation, was found to be:

34 per cent for a mean fibre concentration of 310 million fibres per litre
51 per cent for a mean fibre concentration of 160 million fibres per litre
44 per cent for a mean fibre concentration of 40 million fibres per litre
60 per cent for a mean fibre concentration of 9 million fibres per litre

6.2 Based upon the analysis by one laboratory using four analysts, of fifteen separate aliquots of water taken from the same raw water sample, the within-laboratory precision of the method, expressed as relative standard

deviation, was found to be 25 per cent for a mean fibre concentration of 67 million fibres per litre.

6.3 The accuracy of the method has not been directly investigated, as there are no standards available as yet containing known numbers of asbestos fibres per unit volume of water.

6.4 Indications are that the method has approximately the same relative accuracy as two other methods using transmission electron microscopy, in inter-laboratory comparison tests.

6.5 Since a variable fraction of chrysotile and amphibole fibres may not give identifiable SAED patterns, e.g. due to fibre orientation and size limitations, results given by the method may be biased on the low side.

6.6 In the case of amphiboles, an over-estimation of the mass concentration may be made because of the manner in which fibre volumes are calculated.

7. Interferences and Limitations

7.1 The presence of extraneous particulate material in the sample can, in some instances, cause difficulties in counting and identifying fibres, e.g. by obscuration of the fibres.

7.2 During the sample preparation procedure, particularly for those samples which require low-temperature oxygen plasma treatment, losses of fibres may occur, or break-up of fibres during ultrasonic treatment might produce an increase in the number of fibres actually present.

7.3 Certain mineral fragments which conform to the definition of a fibre may be reported as asbestos, e.g. hornblende or halloysite. Chrysotile fibres are identified by morphology, subject to verification of a proportion by selected area electron diffraction. This procedure reduces the risk of identifying non-asbestos fibres, as chrysotile.

7.4 If, in special cases and studies, more rigorous identification of asbestos is required than is possible by the method described, it is recommended that, in addition, elemental analysis of fibres be carried out by energy or wavelength dispersive x-ray techniques. This procedure requires the use of further complex instrumentation and is not included in the interim method described.

8. Sampling

8.1 Volume of sample - Approximately 1 litre. The sample aliquot taken for analysis may vary from a minimum of 5 ml, to 50 ml using a filter 25 mm in diameter for filtration, and from a minimum of 50 ml, to 500 ml if a filter 47 mm in diameter is used for filtration.

8.2 Sample container - The water sample container should be a polyethylene bottle of 1 litre capacity, preferably new, fitted with a plastic screw cap.

8.3 Preservation - The sample should not be allowed to freeze, should preferably be kept cool and in the dark and should be filtered as soon as possible after collection. If the sample cannot be filtered within 48 hours after collection, algal and bacterial growth may be inhibited by adding 1 ml of a 2.7% per cent solution of mercuric chloride per litre of sample to give a concentration of 20 ppm of mercuric ion.

8.4 Methods of Sampling

8.4.1 General considerations for water sampling apply to the sampling of water for asbestos determination. The sampling site is selected in accordance with the purpose for which the asbestos determinations are required. Consideration should be given to the possibility of variations in fibre size distribution and concentration levels with water temperature gradient, currents and weather conditions.

8.4.2 The sample container should be rinsed at least twice with the water from which the sample is to be taken, immediately prior to sampling. For tap samples, water should be allowed to run at full flow for at least 2 minutes prior to sampling and then reduced in flow as necessary to obtain the sample.

8.4.3 As the water sample must be mixed by shaking immediately prior to analysis, the container should not be filled completely.

9. Laboratory and Cleaning Requirements

9.1 The determination of asbestos must be carried out in an area as free as possible from asbestos contamination. Laboratory floors, ceilings, wall partitions, benching, etc., should be constructed from asbestos-free materials. Materials containing asbestos should not be taken into the laboratory. Special regard must be paid to clothing, especially footwear. It is recommended that the air supply to the laboratory be passed through a 0.3 µm absolute filter.

9.2 All glass and plastic ware used in the analysis, must be thoroughly cleaned. A cleaning procedure which has been found to be effective is as follows: Rinse the ware using distilled water. Place the ware in detergent solution and clean by means of an ultrasonic bath. Rinse the ware thoroughly with filtered distilled water and then once with filtered denatured ethanol. Cover the openings of filter funnels, beakers, etc., with Parafilm, replace lids on petri dishes and cap vials if these are not to be used immediately. As an additional aid in cleaning, an acid rinse, using a solution of nitric or hydrochloric acid may be incorporated into the washing procedure prior to the initial rinse with distilled water.

9.3 Sample containers should be periodically checked for contamination by filling representative containers, selected from the batch to be used, with filtered distilled water, treating for approximately 15 minutes in an ultrasonic bath and taking aliquots of the contents through the method. If a container is found to be contaminated, the batch should either be rejected for use or each container to be used should be cleaned (9.2). Representative containers should then again be checked for contamination.

10. Instrumentation

10.1 Transmission Electron Microscope with an accelerating voltage of 100 KV, a resolution of 0.5 - 1.0 nm, a magnification range of 300X to 100,000X and a binocular attachment with a magnification of about 10X. The microscope should be capable of carrying out selected area electron diffraction (SAED). A means should be available for determining the lengths and widths of fibres, e.g. concentric circles of known diameters on the fluorescent screen, a calibrated graticule in the binocular eyepiece.

10.2 Low Temperature Plasma System with purified oxygen supply for use as required to remove insoluble organic material from samples. The reaction chamber should be large enough to allow the sample vial to be placed in it in an upright position.

10.3 Vacuum Evaporator with rotating sample stage for carbon coating Nuclepore filters.

10.4 Ultrasonic Bath, 50-55KHz, for dispersing ash and for cleaning glass and plastic ware.

10.5 Ultrasonic Probe Assembly, 20 KHz, minimum power 20W, to aid in producing homogeneous ash suspensions.

10.6 Vacuum Pump, to generate a vacuum up to 500 mm Hg for sample filtration.

10.7 Analytical Balance, readability to 0.1 mg, for weighing UICC asbestos samples.

10.8 Calculator, capable of being programmed, for convenience in processing analytical data.

11. Apparatus and Reagents

11.1 Modified Jaffe Wick Washer for use in dissolving Nuclepore filters. An assembly which has been found to be satisfactory is illustrated in Figure 1 and consists of a glass petri dish, 60 mm in diameter, with cover, a stack of approximately twenty Whatman 40 (or equivalent) filter papers, 4.25 cm in diameter, and a minimum of three squares of 200 mesh copper or stainless steel sheet screening, 5 to 10 mm per side, used as microscope grid supports.

11.2 Electron Microscope Grids, copper. 200 and 400 mesh standard or location grids. Grids may be Formvar-coated. Carbon-coated grids are required for determination of the camera constant and for preparation of UICC samples.

11.3 Membrane Filters

Nuclepore, 0.1 µm pore size, 25 mm in diameter, or an equivalent filter, for filtering water samples and ash suspensions. Available from Nuclepore Corp., Pleasanton, California, and from Sargent-Welsh Scientific of Canada Ltd., Weston, Ontario.

Nuclepore, 0.1 µm pore size, 47 mm in diameter, or an equivalent filter, for filtering water samples.

Millipore, type AA, 0.8 µm pore size, 25 mm in diameter, or an equivalent filter, to be used as a backing filter for filtering water samples. Available from Millipore Corp., Bedford, Massachusetts and from Millipore Ltd., Mississauga, Ontario.

Millipore, type AA, 0.8 µm pore size, 47 mm in diameter, or an equivalent filter, to be used as a backing filter for filtering water samples.

Millipore, type GS, 0.22 µm pore size, 25 mm in diameter, or an equivalent filter, for filtering water samples to be ashed.

Millipore, type GS, 0.22 µm pore size, 47 mm in diameter, or an equivalent filter, for filtering water samples to be ashed.

11.4 Filtering Apparatus

Filtration assembly, with frit support and having a funnel with parallel sides, for filters 25 mm in diameter. Used for filtering water samples and ash suspensions. Millipore Corp. Cat. no. XX1002500, or an equivalent assembly.

Filtration Assembly, with filter support, for filters 47 mm in diameter, to be used to filter water samples, ash suspensions, solutions and distilled water. Millipore Corp. Cat. no. XX1504700, or an equivalent assembly.

Filtering Flask, for use with filtering apparatus, 1000 ml.

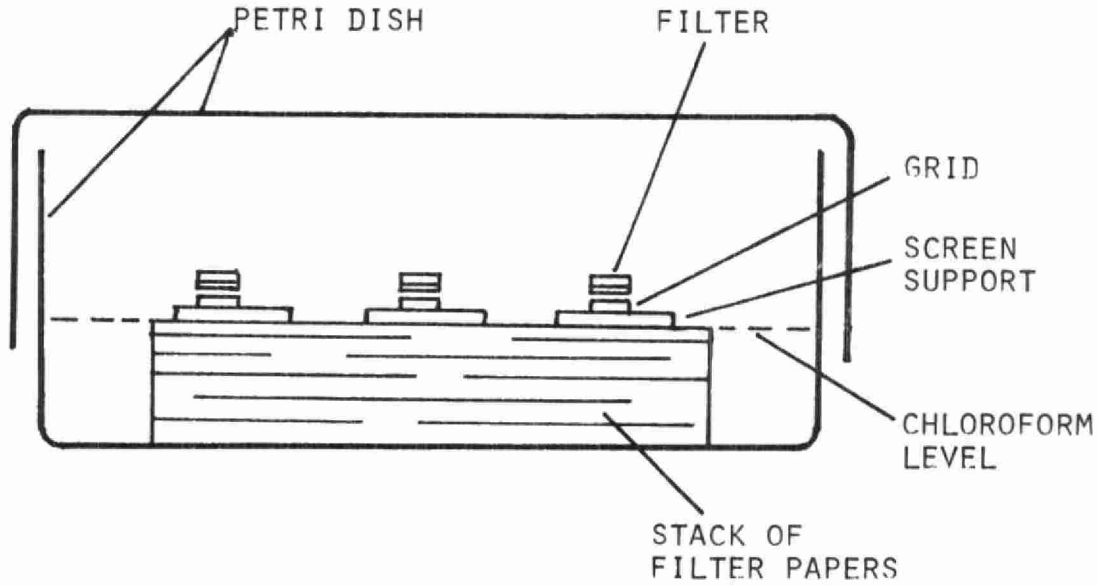
11.5 Carbon Rods, spectrographically pure, for carbon coating Nuclepore filters.

11.6 Carbon Rod Sharpener, compatible with carbon rods, above, for sharpening them to a neck 1 mm in diameter and a minimum of 5 mm in length.

11.7 Carbon Grating Replica, e.g. with 2160 lines per mm, for calibrating the magnification settings of the electron microscope.

11.8 Gold Wire, 100 per cent, approximately 0.2 mm in diameter, for calibrating the camera constant for SAED analysis.

(A) WASHER ASSEMBLY



(B) PRINCIPLE OF METHOD

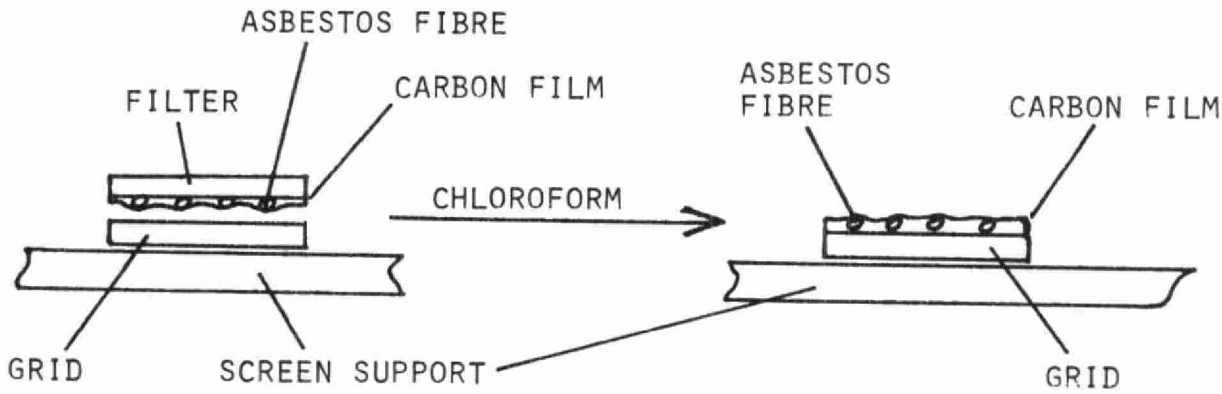


FIGURE 1. MODIFIED JAFFE WICK WASHER AND PRINCIPLE OF METHOD

11.9 Protractor, accurate to 1^{o}, for use in measuring angles.

11.10 Scalpel or Fine Scissors for cutting filter sections.

11.11 Tweezers, flat tipped for handling filters and precision, for handling microscope grids.

11.12 Parafilm, for protecting cleaned glassware from contamination.

11.13 Scotch Magic Transparent Tape, or a similar tape, for securing filters for carbon coating.

11.14 Microsyringe, 10 µl, for dropping chloroform onto microscope grid and filter section.

11.15 Beakers, 250 ml, 500 ml and 1000 ml capacity.

11.16 Graduated Cylinders, 10 ml, 50 ml and 500 ml capacity.

11.17 Pipettes, 5 ml, 10 ml, 25 ml and 50 ml capacity.

11.18 Glass Vials, 30 mm in diameter and 80 mm in length, with caps, for use in the removal of insoluble organic material.

11.19 Petri Dishes, disposable, 60 mm in diameter, for storing and coating filters.

11.20 Mercuric Chloride Solution. Dissolve 2.71 g of mercuric chloride, reagent grade, in 100 ml of distilled water and filter through a 0.1 µm pore size Nuclepore filter. Used as a preservative for water samples when required.

11.21 Chloroform, spectro grade, for dissolving Nuclepore filters.

11.22 Acid Solution, for cleaning glass and plastic ware.

Add one part of nitric acid (approximately 70%), reagent grade, to two parts of distilled water.

Add one part of hydrochloric acid (34 - 38%), reagent grade, to one part of distilled water.

Either acid solution may be used.

11.23 Detergent Solution. Add 20 ml of Decon-75 detergent to 1 litre of distilled water. This solution has been found to be satisfactory for cleaning glass and plastic ware.

11.24 Denatured Ethanol, filtered through a 0.1 µm pore size Nuclepore filter, for cleaning glass and plastic ware.

11.25 Distilled Water, filtered through a 0.1 µm pore size Nuclepore filter, for cleaning purposes, for diluting samples and for preparing ash suspensions.

11.26 UICC* Standard Reference Samples of Asbestos, for use as standards in characterizing asbestos: Chrysotile B, crocidolite, amosite and anthophyllite.

11.27 Molybdenum trioxide, MoO_3, reagent grade, for use in calibrating electron microscopic image rotations.

12. Calibration of the Transmission Electron Microscope.

12.1 To size microscope grid openings and size and classify asbestos fibres in the electron microscope, it is necessary to perform the following calibrations:

* Union Internationale Contre Le Cancer.

12.1.1 Magnification Calibration. The concentric circles on the fluorescent screen and the graticule of the binocular attachment should be calibrated by means of a carbon grating replica. Use of the binocular attachment on most microscopes involves tilting the fluorescent screen with consequent distortion of the image. Therefore, care must be taken to compensate for this in calibrating the graticule.

12.1.2 Determination of the Camera Constant. The constant should be determined by using an electron diffraction standard, e.g. a gold film which has been evaporated onto a carbon-coated electron microscope grid.

12.1.3 Determination of the Relative Angle of Rotation between the Bright Field Image and the SAED Pattern. The angle should be determined by using a substance, e.g. molybdenum trioxide, having a recognizable feature in a known crystallographic direction.

13. Application of UICC Reference Samples to the Identification of Asbestos Fibres.

13.1 To identify asbestos fibres in water samples on the basis of their SAED patterns, it is convenient to compare the patterns with those obtained from well-characterized asbestos fibres. For this purpose, UICC standard reference samples of asbestos are recommended because they are of a high degree of purity and are extensively used for medical and analytical research.

As some degree of subjectivity may be involved in assigning a fibre to a particular class on the basis of its SAED pattern, the analyst should be familiar with the appearance of a variety of patterns given by asbestos and other fibres likely to occur in water samples. Published micrographs of SAED patterns

of chrysotile, amphiboles and related minerals are useful for information and comparison purposes.

13.2 To prepare a given UICC sample, e.g. chrysotile B or crocidolite, for microscopic examination, disperse approximately 1 mg of the sample in 200 ml of filtered distilled water contained in a beaker, by means of an ultrasonic bath. Dilute 5 ml of the suspension to 100 ml with filtered distilled water and re-disperse, to obtain a final concentration of approximately 0.25 mg/l. Using a microsyringe, deposit an aliquot of the latter suspension, 1 to 5 µl in volume, onto a carbon-coated electron microscope grid. Dry the suspension on the grid in air, insert the sample into the microscope and examine several fibres by means of the binocular attachment at an electron microscope magnification setting of 20,000X. Examine and obtain micrographs of SAED patterns from several of the fibres.

13.3 The following characteristics of UICC fibres and SAED patterns should be noted for reference and comparison purposes.

13.3.1 Chrysotile

a) The tubular appearance of the fibres.
b) The spacing of the layer lines of the SAED patterns should correspond to a value of 5.3 Å.
c) The unique streaks in the first layer lines.
d) The triple set of double spots in the second layer lines.
e) The angle between the fibre axis and the layer lines. When corrected for the relative rotation between the bright field fibre image and its SAED pattern, the angle should be 90 degrees.

13.3.2 Amphiboles

a) The arrangement of diffraction spots along the layer lines. Some streaking may occur.

b) The spacing of the layer lines of the SAED patterns. As for chrysotile, this should correspond to a repeat of 5.3 Å.

c) The angle between the fibre axis and the layer lines should be 90 degrees, after correction for the relative rotation between the bright field fibre image and its SAED pattern.

14. Procedure

14.1 Sample Preparation

14.1.1 Centre a 0.1 µm pore size Nuclepore filter, 25 mm in diameter, shiny surface up, with a 0.8 µm pore size Millipore filter, 25 mm in diameter, as a backing filter, on the frit support of a clean filtration assembly.

14.1.2 Apply vacuum to hold the filters in position, and clamp the funnel in place.

14.1.3 Bring the filtering flask to atmospheric pressure by releasing the vacuum.

14.1.4 Agitate the 1 litre plastic container containing the sample for at least 2 minutes to produce a homogeneous suspension.

14.1.5 Using a pipette or graduated cylinder, transfer the entire volume of sample to be filtered (minimum volume 5 ml), into the filter funnel and then apply the vacuum. If the volume of sample to be filtered exceeds the capacity of the funnel, transfer a sufficient amount to nearly fill the funnel. Apply the vacuum and add the remainder to the funnel in such a manner that the level in the funnel during the addition is maintained above the 15 ml mark.

Note 1. Adherence to these instructions is essential in order that the particulate material in the volume of sample taken for analysis be deposited uniformly on the filter surface.

Note 2. The volume of sample to be filtered depends upon the asbestos concentration, the total particulate concentration and the filter size. The minimum volume of sample that can be filtered and give an even deposition of particulate matter including asbestos, on the filter surface is 5 ml for a filter 25 mm in diameter. The maximum volume of sample that can be conveniently filtered is approximately 50 ml.

Note 3. While the sample filtration and preparation procedures are described for filters 25 mm in diameter, equivalent filters of 47 mm diameter, together with compatible filtration assemblies, may be used. In this case the minimum sample volume that can be uniformly filtered is 50 ml, and the maximum volume that can be conveniently taken is 500 ml.

14.1.6 After filtration, release the vacuum and remove the funnel.

14.1.7 Observe the filter for the presence of particulate material. If the sample is high in solids content so that the particulate loading on the filter is judged to be sufficient to interfere with the subsequent electron microscopic examination, prepare another filter by the same procedure, reducing the volume of sample taken for filtration, if possible. If the particulate loading is considered to be excessive after filtration of the minimum volume, filter another aliquot of the sample, following the procedure for removal of insoluble organic material, (14.3), if applicable. Otherwise, dilute the sample as necessary with filtered distilled water and repeat the sample preparation procedure.

14.1.8 If the particulate loading on the Nuclepore filter is judged to be acceptable, transfer the filter by means of tweezers, deposit side up, to a petri dish and secure in place by taping the filter near the margin.

14.1.9 Place the dish containing the filter in a vacuum evaporator and position the carbon rods so that the neck of the sharpened rod is located at a distance of about 7 cm or greater from the filter. With the filter rotating and following manufacturer's instructions, deposit a layer of carbon, approximately 300 Å in thickness, on the filter. Evaporate the carbon in short bursts with a pause between each burst to avoid overheating the filter.

14.1.10 Remove the dish containing the filter from the vacuum evaporator and by means of a scalpel or fine scissors, cut three sections, each approximately 3 mm x 3 mm, from the carbon-coated filter. Avoid cutting the sections near the margin of the filter. It is convenient to leave the remainder of the filter in the petri dish in case additional sections are required for analysis.

14.1.11 Place three 200 mesh electron microscope grids in the modified Jaffe wick washer, one on each square of screening.

14.1.12 Place a drop of chloroform, using a micro-syringe, on one of the grids and immediately place a filter section, with the carbon layer facing down, onto the grid. Place a drop of chloroform, approximately 10 µl in volume, onto the filter section. Repeat

this procedure for each of the remaining two grids and filter sections.

14.1.13 Pour chloroform carefully down the inner wall of the petri dish of the modified Jaffe wick washer until the level rises to the top filter of the filter stack. Replace the cover on the washer and let stand until the Nuclepore filter material is dissolved. A 12 to 24 hour standing period is usually required. Ensure that the chloroform does not evaporate completely from the washer during the period of standing by adding chloroform from time to time as required.

14.1.14 Allow the prepared grids to dry at room temperature prior to storage or electron microscopic examination.

14.1.15 Discard the Whatman filters and the sheet screening of the washer.

14.2 Electron Microscopic Examination

14.2.1 To obtain a result that is representative of the distribution of fibres on the filter within a realistic time limit, at least 2 of the prepared grids are analyzed and at least 4 and not more than 20 grid openings are examined for each sample. Where possible, a minimum of approximately 100 fibres are counted, sized and classified. If the fibre density on the grids is low (less than 5 fibres per grid opening on the average), the fibres in 20 grid openings are counted, sized and classified. The number of grid openings examined should be evenly distributed among

the grids analyzed, subject to the limitation that all fibres in each grid opening must be enumerated.

14.2.2 The total number of grid openings examined per sample may be increased if analytical results of better precision are required than can be achieved by following the procedure outlined in 14.2.1.

14.2.3 If a fibre density greater than approximately 50 fibres per grid opening for a 200 mesh grid is found, the electron microscopic examination becomes unduly arduous. If this occurs, it is recommended that the sample be reprepared, either by filtering less volume, if possible, or by cutting additional sections from the original carbon-coated filter and using 400 mesh grids in the method in place of the 200 mesh grids. The latter alternative, however, is not recommended, if many fibres are present which are relatively long with respect to the dimensions of a 400 mesh grid opening. The sample preparation and grid examination procedures described are applicable to grids of either mesh.

14.2.4 If excessive fibre densities, e.g. greater than approximately 300 fibres per grid opening for a 200 mesh grid, are found, so that the fibres cannot be reliably enumerated, the grids are rejected for analysis. In this case, the sample may be diluted prior to filtration with sufficient filtered distilled water to reduce the fibre concentration to an acceptable level.

14.2.5 Select a grid prepared for analysis and by means of tweezers, place it into the specimen holder with the particulate side facing

down and insert the holder into the specimen chamber of the transmission electron microscope.

14.2.6 Observe the grid at a magnification of about 500X to check for uniformity of particulate deposition, breakage of the carbon film and the presence of undissolved Nuclepore filter material. Reject the grid for analysis if the particles appear to be non-uniformly distributed or if a majority of the grid openings are cracked or broken. Filter material, if present, may be dissolved by further treatment of the grid in the modified Jaffe wick washer.

14.2.7 If the grid is found to be acceptable for analysis, choose a pre-designated or randomly selected intact grid opening. Do not examine openings near the circumference of the grid.

14.2.8 Determine the lengths of two adjacent sides of the grid opening by means of the calibrated measuring device on the microscope and record the measurements.

14.2.9 Increase the magnification setting to 20,000X.

14.2.10 At this magnification, the field of view is considerably smaller than a grid opening, and therefore the grid opening should be scanned in an established pattern to allow all the fibres in the entire grid opening to be counted and also to avoid counting the same fibre twice. Start the scan by positioning the field of view at a corner of the grid opening and scan from one edge of the corner along or to the adjacent edge of the corner by means of one translation control. On completion of the scan, move the field of view perpendicularly to the first scan

approximately the width of the field and again scan from edge to edge on a line parallel to the first scan. Continue viewing in this manner until the entire grid opening has been scanned.

14.2.11 Count the fibres, excluding those of obvious biological origin, by recording as one fibre:

- each separate fibre
- each fibre of a group of fibres intersecting one another
- each fibre intersecting the left-hand grid bar of the opening being examined
- each fibre intersecting the foremost grid bar of the opening being examined

14.2.12 Do not count more than once any fibre which extends into more than one grid opening. Record any masses of fibres present, but do not count them as fibres.

14.2.13 Measure the lengths and widths of the fibres by means of the calibrated measuring device on the microscope and record the measurements. Do not measure fibres that intersect a grid bar.

14.2.14 Examine a fibre with the binocular attachment to determine whether or not the fibre has a tubular morphology. If the fibre has a tubular morphology, record it as such.

14.2.15 Examine the fibre by SAED by selecting a suitable camera length setting and aperture, centring the aperture on the image or a portion of the image of the fibre and switching the microscope to the diffraction mode. Focus the diffraction pattern. Observe the pattern with the binocular attachment. As the quality of the pattern obtained depends

upon fibre size, orientation and the degree of interference from adjacent particles, try to obtain the best possible pattern by adjusting the position of the fibre with respect to the aperture. The duration of the pattern observed may be brief and the analyst must be prepared to note its characteristics quickly.

Note 4. To improve the distinctness of SAED patterns, especially those obtained from small fibres, it is advisable to select a short camera length setting and a small diffraction aperture.

14.2.16 If a diffraction pattern exhibiting all of the characteristics of patterns given by UICC chrysotile (13.3.1) is obtained, record the fibre as being chrysotile, identified by SAED. If the pattern obtained exhibits the characteristics of those given by UICC amphibole asbestos (13.3.2), record the fibre as being an amphibole.

14.2.17 If the fibre gives an SAED pattern which can be characterized as not being that of chrysotile or amphibole, record the fibre as being non-asbestos.

14.2.18 If the fibre gives a partial or no SAED pattern, record the fibre as being ambiguous. A partial pattern is considered to be one which lacks sufficient characteristics to enable the fibre to be classified as asbestos or non-asbestos.

14.2.19 In the same manner, classify all the fibres remaining in the grid openings to be examined by their SAED patterns, except that fibres with a tubular appearance are examined by SAED until approximately 5% of the total number

of those estimated to be present have given patterns characteristic of chrysotile. At this point, provided that no such fibre has been classified as an amphibole or a non-asbestos fibre, consider all fibres with a tubular appearance as being chrysotile and do not continue SAED analysis on the remainder of these fibres.

14.2.20 If desired for the purposes of a particular analysis, chrysotile fibres may be identified on the basis of their SAED patterns only.

14.2.21 Continue to select grid openings from the grids to be examined on a random or pre-designated basis. Examine the grid openings, following the electron microscopic examination procedure, until at least approximately one hundred fibres, if possible, have been counted, sized and classified in the required number of openings (14.2.1).

14.2.22 In order that meaningful results be obtained from the method, the fibre counts from the grid examinations for each sample should be evaluated statistically. A suggested statistical treatment is given in section 17.

14.3 Sample Preparation - Removal of Insoluble Organic Material

14.3.1 Insoluble organic matter, such as algae, plankton, etc. may be present in some samples in sufficient amounts to interfere with fibre counting. In these cases, the material may be removed by treatment in a low temperature oxygen plasma system, following the procedure given below.

14.3.2 Place a 0.22 µm pore size type GS Millipore filter, or its equivalent, 25 mm or 47 mm in

diameter, into the appropriate filtration apparatus.

Note 5. A Millipore membrane filter has been specified because it has a relatively high porosity, is easily oxidized and leaves a low amount of ash. A 0.1 µm pore size Nuclepore filter may also be used, if the sample aliquot can be filtered through it.

14.3.3 Agitate the water sample for at least 2 minutes to obtain a homogeneous suspension.

14.3.4 Transfer a known volume of the sample by means of a pipette into the filter funnel and filter using vacuum.

14.3.5 After filtration, rinse the inside of the funnel with a small amount of filtered distilled water.

14.3.6 Release the vacuum and carefully transfer the filter, by means of tweezers, to a glass vial.

14.3.7 Place the vial and contents in an upright position in the reaction chamber of a low temperature oxygen plasma system.

14.3.8 Reduce the contents of the vial to an ash by operating the plasma system according to manufacturer's directions. Use a controlled evacuation procedure as for powdered samples and set the power and oxygen flow to maintain a low temperature within the reaction chamber, e.g. less than 100°C.

Usually several samples may be treated at the same time, depending upon the capacity of the reaction chamber.

14.3.9 Maintain the plasma until the contents of the vial are judged to be completely reduced to an ash. Usually this requires a period of 6 to 8 hours. Extinguish the plasma and turn off the plasma system, leaving the reaction chamber under vacuum. When the chamber has cooled to room temperature, release the vacuum gradually to avoid loss of ash.

14.3.10 Remove the vial from the chamber and pipette 10 ml of filtered distilled water into the vial. Cap the vial and place it in an ultrasonic bath for a period of 15 minutes to disperse the ash.

14.3.11 Examine the suspension visually and if it is judged that the ash has not been entirely dispersed, remove the cap from the vial, insert the microtip of an ultrasonic probe assembly into the suspension and disperse the ash for 30 seconds at a low power setting (up to an output of approximately 0.5 W/ml of suspension).

14.3.12 Immediately transfer the entire volume of the suspension into a filtration assembly prepared according to 14.1.1, 14.1.2 and 14.1.3. Apply the vacuum and proceed as described in the sample preparation procedure from 14.1.6.

Note 6. Filtration of the ash suspension through a Nuclepore filter 25 mm in diameter will yield a higher fibre density on the filter surface than filtration through a filter 47 mm in diameter. If the larger filter is to be used, the ash suspension must be diluted to a minimum volume of

50 ml with filtered distilled water and re-dispersed in an ultrasonic bath prior to filtration.

14.4 Blank Level Determination

14.4.1 Determine blank levels for chrysotile and amphibole asbestos, as the average number of fibres per grid opening, for a sample or batch of samples by taking an aliquot of filtered distilled water through the method, using a filter of the type representative of that used for sample filtration. Take a 25 ml aliquot if a filter 25 mm in diameter is used and a 200 ml aliquot for a filter 47 mm in diameter.

14.4.2 If the blank level is found to be excessive, the source of contamination must be found and corrected. The possibility of filter contamination should be taken into account when high blank levels are encountered.

14.4.3 Using clean room facilities and cleaning procedures as outlined in 9.1, 9.2 and 9.3, the level of asbestos contamination (usually chrysotile) should not exceed 4 fibres in 20 grid openings of a 200 mesh transmission electron microscope grid.

15. Presentation of Results

15.1 Report the concentration of chrysotile in million fibres per litre and its precision.

15.2 Report the concentration of amphibole asbestos in million fibres per litre and its precision.

15.3 Report the total asbestos concentration in million fibres per litre as the sum of the concentration of the chrysotile and amphibole fibres. Report the precision of the total asbestos concentration.

15.4 Report the results to the nearest 0.2, 1 and 5 million fibres per litre for concentrations in the range less than 1, 1 to 10, 10 to 100 million fibres per litre, respectively, and to 2 significant figures for concentrations greater than 100 million fibres per litre.

15.5 Report the estimated mass concentration of chrysotile in micrograms per litre.

15.6 Report the estimated mass concentration of amphibole fibres in micrograms per litre.

15.7 Report the estimated mass concentration of asbestos in micrograms per litre as the sum of the estimated mass concentrations of chrysotile and amphibole asbestos.

15.8 Record the fibre lengths to the nearest 0.1 µm and the widths to the nearest 0.02 µm.

15.9 Record the aspect ratio of each fibre.

15.10 The data obtained may be reported in the form of length, width, aspect ratio and mass distribution tables and graphs.

15.11 Report the detection limit, or limits, in million fibres per litre.

15.12 Report the number of fibres counted in the following categories:

chrysotile
amphibole
non-asbestos
ambiguous

15.13 Include in the report the analyst's observations on the sample, e.g. the presence and amounts of fibre masses and extraneous material.

16. Calculations

16.1 Determine the fibre concentration of chrysotile or amphibole asbestos from the following formula:

$$C = \frac{(\overline{X} - b) \times Af}{Ag \times V \times 1000}$$

where C = concentration in million fibres per litre

$\overline{X}$ = average number of chrysotile or amphibole fibres per grid opening

b = average number of chrysotile or amphibole fibres per grid opening found for the blank determination

Af = effective filtration area of the Nuclepore filter, in um^2

Ag = average area of the grid openings examined, in um^2

V = volume of the original water sample filtered, in ml

16.2 Estimate the mass concentration of chrysotile from the following formula:

$$Mc = \frac{\pi \times C \times d}{n} \sum_{i=1}^{n} \left[Li \times \left(\frac{Wi}{2} \right)^2 \right]$$

where Mc = estimated mass concentration of chrysotile in µg/l

C = chrysotile concentration in million fibres per litre

d = density of chrysotile, which is taken to be 2.5 g/cm^3

n = number of chrysotile fibres measured

L = length of each chrysotile fibre, respectively, in µm

W = width of each chrysotile fibre, respectively, in µm

16.3 Estimate the mass concentration of amphibole asbestos from the following formula:

$$Ma = \frac{C \times d}{n} \sum_{i=1}^{n} (Li \times Wi^2)$$

where Ma = estimated mass concentration of amphibole in µg/l

C = amphibole concentration in million fibres per litre

d = density of amphibole fibres, which is taken to be 3.25 g/cm^3

n = number of amphibole fibres measured

L = length of each amphibole fibre, respectively, in µm

W = width of each amphibole fibre, respectively, in µm

16.4 Determine the detection limits for chrysotile and amphibole asbestos from the following formulae:

16.4.1 for a blank level greater than zero

$$DL = \frac{2b \times Af}{Ag \times V \times 1000}$$

where DL = detection limit for chrysotile or amphibole asbestos in million fibres per litre

b = average number of chrysotile or amphibole fibres per grid opening found for the blank determination

Af = effective filtration area of the Nuclepore filter, in μm^2

Ag = average area of the grid openings examined for the sample, in μm^2

V = volume of the original water sample filtered, in ml

16.4.2 for a blank level of zero

$$DL = \frac{Af}{N \times Ag \times V \times 1000}$$

where DL = detection limit for chrysotile or amphibole asbestos in million fibres per litre

Af = effective filtration area of the Nuclepore filter, in μm^2

N = number of grid openings examined for the sample

Ag = average area of the grid openings examined for the sample, in μm^2

V = volume of the original water sample filtered, in ml

17. Suggested Statistical Evaluation of Grid Fibre Counts

17.1 Since the nature of the fibre distribution on the sample filter, resulting from the method of filtration, has not been fully determined, the fibre distribution obtained on the electron microscope grids for each sample should be tested statistically against an assumed distribution and a measure of the precision of the analysis should be provided.

17.2 In view of the sample preparation procedures used, an approach is to consider the fibres to be uniformly and randomly distributed on the sample filter and grids. A suggested means for confirming this assumption is given below.

17.3 Determine, using the chi-square test, whether the total number of fibres found in individual grid openings are randomly and uniformly distributed among the openings, by the following formula:

$$X^2 = \sum_{i=1}^{N} \frac{(ni - npi)^2}{npi}$$

where X^2 = the chi-square statistic

ni = observed total number of fibres in each grid opening, respectively.

n = total number of fibres found in N grid openings

N = number of grid openings examined for the sample

pi = the ratio of the area of each respective grid opening to the sum of the areas of the grid openings examined.

If the value for X^2 exceeds the value listed in statistical tables for the 0.001 significance level with N - 1 degrees of freedom, the fibres are not considered to be uniformly and randomly distributed. In this case, it is advisable to try to improve the uniformity of fibre deposition, if possible, by filtering another aliquot of the sample and repeating the

analysis. For samples for which X^2 exceeds the listed value, only an estimate of the range of the fibre concentration can be made. The upper and lower range values are obtained by substituting, respectively, the highest and lowest grid opening fibre counts for the $(\overline{X} - b)$ term in the equation given in 16.1.

17.4 If uniformity and randomness of fibre deposition on the sample grids has been demonstrated as in 17.3, the 95% confidence interval about the mean fibre counts for chrysotile, amphibole and total asbestos may be determined using the following formulae:

(1) $$Sc = \left[\frac{N \sum_{i=1}^{N} X_i^2 - \left(\sum_{i=1}^{N} X_i \right)^2}{N(N-1)} \right]^{\frac{1}{2}}$$

where Sc = standard deviation of the fibre counts for chrysotile

N = number of grid openings examined for the sample

Xi = number of chrysotile fibres in each grid opening, respectively

Obtain the standard deviations of the fibre counts for amphibole asbestos and for total asbestos by substituting the corresponding values of Xi into equation (1).

(2) $$Xu = \overline{X} + \frac{tSc}{\sqrt{N}}$$

(3) $$X_1 = \overline{X} - \frac{tSc}{\sqrt{N}}$$

where Xu = upper value of 95% confidence interval for chrysotile

X_1 = lower value of 95% confidence interval for chrysotile

$\overline{X}$ = average number of chrysotile fibres per grid opening

t = value listed in t-distribution tables at the 95% confidence level for a two-tailed distribution with N-1 degrees of freedom

Sc = standard deviation of the fibre counts for chrysotile

N = number of grid openings examined for the sample.

The values of Xu and X_1 can be converted to concentrations in million fibres per litre by using the formula in 16.1 and substituting either Xu or X_1 for the term $(\overline{X} - b)$.

Obtain the upper and lower values of the 95% confidence intervals for amphibole asbestos and total asbestos by substituting the corresponding values of $\overline{X}$ and Sc into equations (2) and (3).

Report the precision of the analysis, in terms of the upper and lower limits of the 95% confidence interval, for chrysotile, amphibole asbestos and total asbestos. If a lower limit is found to be negative, report the value of the limit as zero.

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